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THE VALUE OF STUDYING THE MUTAGENIC AND EMBRYONIC PROPERTIES OF PESTICIDES FOR TOXICOLOGICAL-HYGIENIC ASSESSMENT

(Znacheniye issledovaniy mutagennykh i embriotoksicheskikh svoystv pestitsidov dlya ikh toksikologo-gigiyenicheskoy otsenki)

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THE VALUE OF STUDYING THE MUTAGENIC AND EMBRYONIC PROPERTIES OF PESTICIDES FOR TOXICOLOGICAL-HYGIENIC ASSESSMENT

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With the rapid development of chemistry a new, previously unknown chemical environment is being created not only for man, but also for all life. Historically accumulated biocenoses are being disturbed. The effect of chemicals becomes a powerful ecological factor in the complex of environmental conditions.

Especially distinguished among chemical substances are pesticides, with which millions of people of all ages come in contact, beginning with the early stages of embryonic development. Contributing to this has been the wide use of pesticides, their circulation in the air, soil and water, in plant and animal organisms (L. I. Medved', 1967). Toxicologists are faced with a new task--the necessity of studying the remote unfavorable effects of pesticides on the human organism which has come into immediate contact with them and on their offspring. The necessity for this kind of research to make a more complete hygienic assessment of pesticides and better regulate their use is being emphasized by many Soviet and foreign authors (I. A. Rapoport, 1963, 1966; S. L. Danishevskiy, A. Ya. Broytman, 1966; N. V. Lazarev, 1966; L. I. Medved' et al., 1967, and many others).

During the process of individual development of the organism, chemical substances penetrating it can cause both hereditary and nonhereditary changes.

It is known that during embryogenesis the sensitivity of the organism to different effects is extremely high, especially in the critical periods, and if the agent does not stop the development of the embryo completely, it can significantly damage it, leading to every kind of embryopathy and revealing an embryotoxic effect (P. G. Svetlov, 1962; A. P. Dyban, 1964). In recent years the embryotoxic properties of several medicines have been noted (E. D. Manevich, 1966), as well as pesticides, including granozane (Ye. A. Goloma, 1962; N. D. Mukhtarova, 1965) and dithiocarbamic acide derivatives (L. V. Martson', 1966; R. N. Ryazanova, 1967; V. Kh. Yenikeyev, 1968, and others).

With damage to the genetic structures of the cell, persistent hereditary changes develop — mutations, found both in sex and somatic cells and at every stage of development of the organism. At the present time more than 500 different hereditary diseases and deformities are known in man (Ye. F. Davidenkova, 1965). It has been suggested that 3/4 of the spontaneous mutations in man now develop from the effect of chemical substances, including pesticides (Fahmy O. G., Fahmy M.J., 1964). Thus, with the ever increasing organic synthesis and growing number of chemical mutagens, the frequency of induced mutations also increases, which can entail a still greater hereditary burden on man.

All this emphasizes the necessity for a systematic study by toxicologists and hygienists of widely used pesticides, as possible agents carrying embryonic and genetic danger to man.

There is almost no data of this kind in relation to man. Systematic study of the mutagenicity of industrial poisons, products of chemical synthesis and widely used pesticides is not being conducted. This is to a certain extent due to a lack of generally accepted methods of studying the genetic effects of chemical substances. There have been individual works with other than an hygienic approach. Here we can cite the discovery of the cytogenetic effect of zoocumarine, granozane and the group of chlororganic insecticides (DDT, heptachlorine, hexachlorine, aldrin, dildrin) on bone marrow cells of

mice and rats (D. S. Markaryan, R. N. Mandzhgaladze, V. I. Vashakidze, 1966; D. S. Markaryan, 1966), as well as that of parachinondioxime on a culture of embryonic fibroblasts and leukocytes of human peripheral blood (Yu. A. Revazova, 1967).

We must point out that although in these and similar works we are talking of mutation changes in somatic cells, there is every basis for considering that they also occur with the same frequency in sex cells. The specificity of the reaction of various biological subjects to the action of chemical mutagens must certainly be taken into consideration, especially in studying these substances on a hygiene plane to determine the danger to man.

It seems to us that in order to attain this goal, besides using laboratory animals, it is also necessary to conduct research on human cells, particularly on a leukocyte culture of peripheral blood. This model allows us to make a direct study of the effect of substances on the chromosome apparatus of cells "in vitro." By using it, chromosome damage "in vivo" can be considered in parallel by the leukocyte karyotype of persons coming in contact with poisonous chemicals.

As is known, chromosome analysis in a leukocyte culture is at present widely used to solve many theoretical and practical questions in medical cytogenetics. It is widely used now for studying the effect of radiation energy, viruses and medicines on the chromosome apparatus of human cells "in vitro" and "in vivo." This method is recommended in foreign literature for inclusion in the medical examination of workers involved with radiation (Normal Amos, et al., 1964).

We shall discuss some data we obtained in tests to find the mutagenic and embryotoxic properties of dithiocarbamic acid derivatives--the fungicides cineb, cirame and maneb.

Possible mutagenic activity of these preparations was tested at larval and adult stages of Drosophila by two methods--CLB and "Meller-5", which take into consideration lethal mutations connected with sex, which develop

under the effect of the agent studied. The substances were tested in various doses (cineb—0.3 LD_{50} and LD_{50}, cirame—2.5 LD_{50} and 1.5 LD_{50}). As the tests showed, cineb and cirame were not mutagenic for the fruit fly, neither at its adult nor larval stages, with either large or small doses. Maneb, despite its chemical similarity to cineb, from which it differs only in the content of manganese instead of zinc, was a weak mutagen for Drosophila, increasing in a small dose (0.5 LD_{50}) the percentage of lethality by twice as much, in comparison with the control (from 0.35% to 0.7%).

In tests on a leukocyte culture of peripheral human blood, cultured according to the Murkhed method, without erythrocytes, with 24-hour exposure of cineb and cirame in doses changing the mitotic activity of cells, an increase in the percent of chromosome aberrations is noted — from 40.0 to 45.3% with 8% in the control. Data is given in Table 1.

We can see from Table 1 that when leukocytes are cultured in whole blood, that is to say together with erythrocytes and other regular elements, leukocytes react more weakly to the effect of cirame, namely, a dose 4 times greater (0.06 µg/ml) than with culturing in plasma (0.015 µg/ml) causes a very small effect, yielding only 14% cells with chromosome aberrations, while culturing leukocytes with cirame in plasma causes 24% aberrant cells. This is subject to further study.

Maneb in a leukocyte culture of whole human blood, with 24-hour exposure in a dose of 30 µg/ml shows a high cytogenetic effect.

These data indicate the potential genetic danger of these fungicides for human chromosomes in a direct effect on the cell, that is when protective mechanisms of the organism are completely eliminated. In connection with this, further study of the effect of these substances on the complete organism of the laboratory animal with maintenance of enzyme, nerve, physical-chemical and other mechanisms and correlations is necessary. Experiments conducted on random bred white mice with acute cirame inoculation (perorally) in doses of LD_{50} (220 mg/kg) and 0.2 LD_{50} (22 mg/kg) detected changes in the chromosome

TABLE 1. THE EFFECT OF CINEB, CIRAME AND MANEB ON CHROMOSOMES OF HUMAN BLOOD LEUKOCYTES "IN VITRO"

Preparation	Dose in µg/ml*	Number of cells studied	% of aneuploid cells	% of cells with chromosome aberrations	Total number of anomalous cells	Number of breaks in one cell
Control	—	100	6	2	8	0.02
Cineb	0.125	116	9.9	35.0	45.3	0.44
Cirame						
a) leukocytes in plasma	0.015	100	16	24	40	0.3
b) leukocytes in whole blood	0.06	50	--	14	--	0.18
Maneb**	30.0	20	--	35	--	

*Dose of substance changing mitotic activity, but not toxic for cells.

**Data is provisional. Chromosome analysis is continuing. The effect of maneb was studied in a leukocyte culture of whole blood.

apparatus of bone marrow cells 24 hours after the effect, expressed in an increase in the number of cells with chromosome damage from 1.8% in the control, to 4.0% and 6.9% in the test.

Thus, the data obtained indicate the coincidence of test results in human cells "in vitro" and cells of mice "in vivo," on the one hand, and the absence of such correspondence with results obtained with insects on the other.

Considering the problems connected with protecting human health, on the basis of our short test we are inclined to feel that Drosophila is not a completely suitable model to expose the genetic danger of pesticides for man, although it is the classic genetic subject which has helped solve many impor-

tant questions of chemical mutagenesis during its 30-year period of development (V. G. Nikiforov, 1965, and others).

With respect to the possibility of transferring experimental data from animals to man, we are here in the same position as all the other experimenters: it is necessary to experiment with animals, but the selection of test animals must be based on the qualitative agreement of biochemical structures and reactions with those in man (L. A. Tiunov, 1967), which will ensure the same direction of response to the effect being studied. It is also necessary to consider the high specificity of chemical mutagens and the comparative specific sensitivity of experimental animals and man, as well as the ability of human and animal cells to react with similar genetic changes to the effect of these agents.

As it does not seem possible to determine the mutagenecity of chemical substances directly on human sex cells, at this stage, lacking adequate techniques, a complex approach would perhaps be best, using several methods "in vitro" and "in vivo." In the first place , as it seems to us, it is necessary to study the possible cytogenetic effect by the leukocyte karyotype of peripheral blood in human cells "in vitro," as well as "in vivo" in persons coming in contact with poisonous chemicals. Later, after finding a similar cytogenetic effect in the laboratory animal, one can limit oneself to given model animals to determine the actual genetic danger of the chemical substance. It is extremely important to find the length of time the cytogenetic effect is maintained in acute and chronic inoculation, as well as under the influence of insignificant small doses of pesticides throughout the entire lifetime of the animal. To study the cytogenetic effect in dynamics in the future, the question will evidently arise of using a method of leukocyte culture of experimental animals, as well as animal sex cells to study chromosome damage.

A second direction in studying possible remote unfavorable aftereffects of pesticides was a study of the effect of the fungicides cineb, cirame and maneb on the embryonic development and generative function of random bred

white rats (weighing 150-200 g). After pregnancy of the animals was established, these preparations were administered to the stomach every other day throughout the entire period of pregnancy. Tested first were high doses, not toxic to the adult organism (0.17-0.20 LD_{50}). When embryotoxicity of the substance was observed, the dose was reduced until results became similar to those in the control. The following indices were considered: the number of yellow bodies and resorbed fetuses, the number of stillborn baby rats, characteristics of postnatal development, survival, etc. Negative indices were combined under the heading: "unfavorable outcome of pregnancy."

TABLE 2. DEPENDENCE OF THE EMBRYOTOXIC EFFECT OF DITHIOCARBAMIC ACID DERIVATIVES ON DOSAGE

Preparation	Substance administered		Unfavorable course of pregnancy in %
	portion of LD_{50}	in mg/kg	
Control	--	--	12.0
Cineb	0.17	300	22.6
	0.03	50	11.5
Cirame	0.20	250	97.8
	0.03	42	22.6
	0.01	14	4.4
Maneb	0.01	50	21.2
	0.005	25	25.0

These data are given in Table 2; it is evident that all three of these dithiocarbamic acid derivatives have embryotoxic properties with large doses; according to the strength of the effect, they can be arranged in the following descending order: cineb, having no effect in a dose of LD_{50}, cirame--in a

dose of 0.01 LD_{50} and maneb, which in a dose of 0.005 LD_{50} still has an unfavorable effect on offspring (25% various disorders in comparison with 12% in the control group).

In studying the gonadotoxic effect of maneb, sexually immature young rats were subjected to the effect of 0.01 LD_{50} for a month. After attaining sexual maturity the animals in the test group were crossed with corresponding controls, the time of the onset of pregnancy considered and characteristics of the sexual behavior of the animals noted, as well as embryonic and postembryonic development. As a result of the tests it was noted that maneb causes damage to the reproductive function and this effect is more pronounced in male gonads. However, these changes were reversible; functional damage was repaired in 3.5 months after the end of the effect.

We can draw two conclusions from our short test studying the mutagenic and embryotoxic properties of the fungicides cineb, cirame and maneb:

1. In experiments on the bone marrow of mice and a leukocyte culture of human peripheral blood, the cytogenetic activity of cineb, cirame and maneb was revealed. Their embryotoxic effect on rats was shown in comparatively high doses, not toxic for the adult organism. This indicates potential danger of these substances according to both indices.

2. Genetic studies for hygienic purposes must be conducted on biological subjects far removed from man, in particular, on insects. The potential and possible actual genetic danger of pesticides can be assessed on the basis of comparing data from tests on human cells *in vitro*; and on the entire organism of laboratory animals.

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